Nutritional value, cellulase activity and prebiotic effect of melon residues (*Cucumis melo* L. *reticulatus* group) as a fermentative substrate

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Summary

Melon residues were used to produce cellulases (EC 3.2.1.4) and to investigate the prebiotic effect in vitro. Peel and seed flours were characterized regarding contents of dietetic fibre, lipids, total protein, cellulose, hemicelluloses and lignin. A kinetic study was carried out in bacteriological greenhouse during 24, 48, 72, 96, 120, 144 and 168 h. In melon seed flour, cellulose at 350 $g \cdot kg^{-1}$, hemicellulose at 79 $g \cdot kg^{-1}$ and lignin at 24 $g \cdot kg^{-1}$ were determined. Results showed, in terms of dietetic fibre and high protein contents, that both seeds and peels have good nutritional value. The highest content of lipids, 246 $g \cdot kg^{-1}$, was found in seeds, compared to only 36.3 $g \cdot kg^{-1}$ in the peel. The best activity for carboxymethyl cellulase in 144 h of fermentation was 1.045 $U \cdot g^{-1}$, while the activity with filter paper after 96 h of cultivation was 0.190 $U \cdot g^{-1}$. Melon seed flour demonstrated to be a good substrate for the growth of bifidobacteria with 8 h for fermentation, and it was tolerant to the action of bile salts at 8 h of fermentation. Melon residues can be taken as a potential prebiotic ingredient and a source of cellulolytic enzymes.

Keywords

melon peel; melon seed; cellulase; prebiotic activity

According to the FAOSTAT database of Food and Agriculture Organization (Rome, Italy), world production of melon presented in recent years an increment of 55 %, increasing from 20 million tons in a cultivated area of 1.1 million hectares in 1999 to 31 million tons in an area of 1.4 million hectares in 2013 [1]. The Brazilian Institute of Geography and Statistics (Sao Paulo, Brazil) showed that in 2011 the harvest of the fruits reached over 44 million tons. Despite this growth, a large amount of fruits is wasted [2].

Usually, the non-edible parts of the melon peel and seeds are completely discarded during processing and habitual consumption, constituting for 8–20 million tonnes per year globally. Therefore, new aspects concerning the use of peel and seeds for the production of food additives, supplements with high nutritional value or application for biotechnology in the production of enzymes and energy, have attracted an increased interest [3].

The peels and seeds contain mainly carbohydrates, proteins and fibres, which enables their use in bread and sweets, increasing their commercial value. Added value to these by-products promotes their application in the food industry. Their important nutritional properties classify them as raw materials so they can be used in a range of market segments.

By the advent of biotechnology and bioprocess, the use of residues for the production of enzymes became viable economically and regarding sustain-

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ability. Production of enzymes from fermented residues was implemented in several industries in recent decades because they are specific, non-toxic, inexpensive and often save raw materials, energy, chemicals and/or water compared to conventional processes [4]. Degradation of lignocellulosic biomass is performed primarily by microbial action, i.e. they utilize it as a source of carbon, energy and nutrients for growth. Bacteria and fungi are commonly used in such bioprocesses. Out of these, filamentous fungi are the main agents involved in residue degradation, which is accompanied by production of extracellular enzymes [5]. The lignocellulosic residues are commonly degraded by an enzyme complex of ligninases, cellulases and hemicellulases [6].

Lignocellulosic residues are agricultural crop residues, which may also originate from the food processing industry and food services. These residues present, in its constitution, lignin and cellulose, together with other components such as hemicelluloses and pectin. They possess a great biotechnological potential for obtaining products of industrial interest, such as enzymes and prebiotic ingredients [7].

Cultivation in solid state is one of important strategies used in industry to optimize production of various enzymes such as cellulases. Among the sectors that benefit from enzyme technology is the food industry, as it faces a growing demand for products with fewer additives and preservatives. In this context, enzymes play a key role in cleaner technologies. Based on a high demand, new functional ingredients are studied regarding prebiotic properties. These substances would stimulate the growth of probiotic bacteria, which are beneficial to the host. Such products have a high added value and can be obtained from unusual sources such as agricultural waste [8].

Peel and seeds of melon may be residues utilizable as food additives with an added value. This study investigated the potential of melon residues as a substrate in semi-solid culture for bioproduction of cellulases and the in vitro prebiotic potential.

MATERIALS AND METHODS

Lignocellulosic residues

The peels and seeds of *Cucumis melo* L. var. *reticulatus* (cantaloupe melon) were obtained from an university restaurant in northeastern Brazil. For the technological process of preparation of flours, seeds and peels were dried in a forced ventilation oven in accordance with the experimental design. The dry material was ground in a food processor and sieved using a sieve of 0.841 mm. A 2^2 factorial design was applied to prepare the powders with 3 centre point repetitions. The influence of drying air temperature (X_1) and drying time (X_2) assayed at three equidistant levels (–1, 0, +1) on the responses (dependent variable) moisture, water activity and yield of the powders was investigated.

The independent variables (factors) were chosen for their importance in food drying. Real values for the factors were 60 °C, 70 °C and 80 °C for temperature and 24 h, 30 h and 36 h for drying time, in a total of 7 runs (Tab. 1). Response surfaces for the design were assessed in terms of the responses water activity, moisture and yield. Water activity was determined using a water activity meter (BrasEq Aqualab, São Paulo, Brazil). Moisture was determined by oven drying at 105 °C to constant weight [9], and the percentage of flour yield was calculated by the ratio between the weight of the dry flour and the weight of the seed in natura.

Characterization of residues

To characterize the products prepared from melon residues, the best condition for the powder production was used, as determined by experimental planning. The following analyses were carried out in triplicate, in accordance with Association of Official Analytical Chemists (AOAC) [9]. Moisture was determined by drying in an oven with air circulation at 50 °C until constant weight (method 976.05). Ash was determined by incinerating the sample in a muffle furnace at temperatures of 500–600 °C for 4 h (method 923.03). Proteins were determined by the Kjeldahl method, the protein content being calculated by multiplying the total nitrogen content by 6.25 (method 976.05). Crude fat as an ether extract was deter-

Tab. 1. Delineation of the 2^2 full factorial design with three central points.

Run order	Levels of coded independent variables		Levels of real independent variables	
	X_1 (temperature)	X_2 (time)	Temperature [°C]	Time [h]
2	–1	+1	60	36
1	–1	–1	60	24
7	0	0	70	30
5	0	0	70	30
6	0	0	70	30
4	+1	+1	80	36
3	+1	–1	80	24

mined using a Soxhlet extractor (method 920.39). For extraction, ether as a solvent was continuously refluxed through the sample for 6 h. After that, the solvent was recovered and then the balloons were weighed, obtaining the amount of lipids by the difference in balloon weight. The method to determine the fibre fractions, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and lignin was according to GOERING and VAN SOEST [10]. Total carbohydrates were calculated by the difference method, summing the values of moisture, crude protein, ash and crude fat (ether extract) and subtracting the sum from 100 [11].

Microstructure analysis

Microstructure was analysed by scanning electron microscopy (SEM) using a TM-3000 Tabletop Microscope (Hitachi, Tokyo, Japan). The images generated were evaluated using secondary electrons accelerated to a voltage of 5.0 kV and digitally captured. The samples were mounted onto metal stubs and subjected to sputtering using carbon tape.

Enzymatic production and enzyme extraction

The residue (melon peel and seed flours) was sterilized by autoclaving at 121 °C for 15 min. The microorganism used in the solid state cultivation (SSC) was the filamentous fungus *Aspergillus oryzae* ATCC 9362. It was inoculated at a spore content of 10^7 spores per gram of solid medium. SSC was conducted at a temperature of 30 °C for 7 days in a Biological Oxygen Demand (BOD) incubator (Thermo Fisher Scientific, Waltham, Massachusetts, USA) withdrawing an Erlenmeyer flask of 250 ml every 24 h.

The culture medium was composed of 5.0 g of the flour, 5.0 ml of the inoculum was added and 10 ml of a nutrient solution (0.1% ammonium nitrate, 0.1% K_2HPO_4, 0.05% NaCl, 0.05% yeast extract and 1.0% urea), with pH adjusted to 5.5. All chemicals and nutrients were obtained from Merck (Darmstadt, Germany).

The fermentation process was initiated at a moisture of 60% and the nutrient saline pH of 5.5. Enzyme extraction was carried out after 24, 36, 48, 72, 96, 120, 144 and 168 h of cultivation using 30.0 ml of sodium citrate buffer (200 mmol·l^{-1}, pH 5.5, Merck) added to the reaction mixture that was shaken at 2.5 Hz at 4 °C for 2 h. Then the extract was clarified by filtration and centrifuged at 1560 ×*g* for 10 min at 4 °C. The supernatant containing the enzyme extract was used for analysis of enzyme activities, reducing sugars and proteins.

Carboxymethyl cellulase activity and filter paper activity

For carboxymethyl cellulase (CMCase, EC 3.2.1.4) activity, the reaction test tube was added 0.5 ml of sodium citrate buffer (50 mmol·l^{-1}, pH 4.8), 0.5 ml enzyme extract and 0.5 ml of carboxymethylcellulose (CMC, 2 g·l^{-1}). The control was added 0.5 ml of the buffer solution and 0.5 ml of CMC (2 g·l^{-1}), and the blank tube contained 0.5 ml of 3,5-dinitrosalicilic acid (DNS) and 0.5 ml of the buffer. All samples were incubated in a water bath at 50 °C for 10 min and then the reaction was stopped by adding 0.5 ml of DNS. All tubes were put into a water bath containing boiling water for 5 min. Then, 4.0 ml of distilled water was added and absorbance was measured at 540 nm using a spectrophotometer Thermo Spectronic (Thermo Fisher Scientific) [12, 13].

The filter paper activity (FPase) was measured using 1.0 ml of sodium citrate buffer (50 mmol·l^{-1}, pH 4.8), 0.5 ml enzyme extract and a cellulose filter paper Whatman No. 1 (1.0 cm × 6.0 cm; Whatman, Maidstone, United Kingdom) [11]. For the reaction control, 1.0 ml of the same buffer solution and 0.5 ml enzyme extract were added. The substrate control contained 1.5 ml of the buffer solution and a strip of filter paper. All tubes were put into a water bath containing boiling water for 5 min. Next, 4.0 ml of distilled water was added and absorbance was measured at 540 nm using a spectrophotometer Thermo Spectronic.

Standard calibration curves for CMCase and FPase were constructed using glucose in concentrations from 0.2 g·l^{-1} to 2.0 g·l^{-1}. A unit of enzymatic activity (U) was defined as the amount of enzyme capable of releasing 1 mol of reducing sugars per minute at 50 °C. The enzymatic activity was expressed in enzyme units per gram of substrate.

In vitro prebiotic potential

The in vitro prebiotic potential of the melon residues was investigated by submerged fermentation by *Bifidobacterium animalis* subsp. *lactis* DSM15954 (Chr. Hansen, Hoersholm, Denmark) at 37 °C for 24 h under anaerobiosis. The cultivation medium contained melon peel and seed as substrates. A prebiotic fructooligosaccharide (FOS) was used as a positive control. An aliquot of 1.0 ml of the liquid metabolite was withdrawn every 2 h, serially diluted (10^0–10^9) and plated on the surface of selective agar for *Bifidobacterium* (BD, Heidelberg, Germany). The plates were incubated in an anaerobic bacteriological jar (Gaspak Jar, Sparks, Maryland, USA) at 37 °C for 72 h and then colony forming units were counted.

Tab. 2. Results of the experimental design for melon seed and peel drying.

Run order	T [°C]	t [h]	Seed powder			Peel powder		
			Y [%]	M [%]	a_w	Y [%]	M [%]	a_w
2	60	36	8.3 ± 0.2^{c}	3.8 ± 0.1^{c}	0.23 ± 0.01^{a}	5.1 ± 0.1^{b}	13.8 ± 0.3^{a}	0.38 ± 0.02^{a}
1	60	24	8.6 ± 0.1^{c}	6.7 ± 0.1^{bc}	0.28 ± 0.02^{a}	5.1 ± 0.1^{b}	14.8 ± 0.3^{a}	0.35 ± 0.01^{a}
7	70	30	25.7 ± 0.2^{b}	4.7 ± 0.1^{ab}	0.26 ± 0.01^{a}	7.4 ± 0.1^{a}	12.9 ± 0.2^{b}	0.36 ± 0.01^{a}
5	70	30	27.1 ± 0.7^{b}	5.9 ± 0.1^{abc}	0.24 ± 0.01^{a}	6.6 ± 0.1^{ab}	13.9 ± 0.2^{ab}	0.37 ± 0.02^{a}
6	70	30	29.1 ± 0.3^{b}	7.9 ± 0.2^{a}	0.29 ± 0.02^{a}	6.7 ± 0.1^{ab}	11.8 ± 0.3^{bc}	0.37 ± 0.01^{a}
4	80	36	40.2 ± 1.0^{a}	3.9 ± 0.1^{c}	0.22 ± 0.01^{a}	6.9 ± 0.1^{ab}	12.7 ± 0.4^{b}	0.35 ± 0.03^{a}
3	80	24	43.4 ± 1.1^{a}	3.6 ± 0.2^{c}	0.24 ± 0.01^{a}	7.5 ± 0.1^{a}	12.9 ± 0.4^{b}	0.35 ± 0.03^{a}

Different lower case letters in the same column indicate a significant statistical difference ($p < 0.05$) in Tukey's test.
T – temperature, t – drying time, Y – yield, M – moisture content on dry basis, a_w – water activity.

Afterwards, identity of the strain was verified by Gram staining [14]. Also, every 2 h of fermentation, the metabolic liquid was evaluated for microorganism resistance to bile salts (Sigma Aldrich, St. Louis, Missouri, USA). In this case, the metabolic liquid was added to a solution containing bile salts at a concentration of 0.3 % and incubated anaerobically at 37 °C for 3 h. Then, the cell suspension was directly assessed by measuring absorbance at 600 nm [15].

Statistical analysis

Results were expressed as mean and standard deviation. Analysis of variance (ANOVA) and mean testing were performed at a 95% confidence interval ($p < 0.05$) using Statistica software v.7 (Statsoft, Tulsa, Oklahoma, USA). All experiments carried out in three replicates.

RESULTS AND DISCUSSION

In order to investigate the influence of the drying air temperature and drying time on moisture, water activity and yield of the powders, a 2^2 factorial design with a triplicate at centre point was used. Regarding the experimental design of the drying process, time and temperature were determining factors, since high temperatures for an extended time could induce decomposition of the phenolic compounds. In a previous study, the same melon residues were evaluated for contents of bioactive compounds and for antioxidant capacity. The melon peel and seed extracts were found to contain significant amounts of gallic acid, catechin and eugenol, and to possess a high antioxidant activity in in vitro assays [16]. For this reason and in order to avoid possible loss of phenolic compounds, it was chosen in the experimental design to reduce the temperature and increase the time to ensure the water loss process as well to achieve a good yield.

Tab. 2 shows the results of the experimental design for melon seed and peel powder. The experimental design allowed to study the best drying conditions to obtain good yield, low water activity and low moisture content in order to comply with technical specifications of the European Union Commission [17] about food additives, which establishes the maximum moisture content of 15.5 %.

The Pareto's chart provides the estimate of a quantitative effect that each variable has on the yield, establishing that these effects are within the confidence interval defined for the statistical analysis (95 %). According to the Pareto chart in Fig. 1 for the performance of melon residues flour, the temperature was the only significant factor, at a 95% significant level, for both seed and peel. The time and interaction did not positively affect the yield during drying.

Benefits of the process included its simplicity, protection from microorganisms and a longer shelf life of the products. However, processing generally causes loss of bioactive compounds in relation to fresh fruits, which reduces health benefits. This is largely due to a decreased antioxidant activity. Nevertheless, different studies indicated that foodstuffs submitted to drying retained most of their bioactive compounds as well as carotenoids and antioxidant activity [18–20].

As already shown in Fig. 1, in relation to the yield, temperature was the most important factor for both seed and peel, with no effect observed for moisture or water activity. The yield of melon seed was significantly higher than that of peel. This is an important parameter from an industrial and technological perspective. As shown in Tab. 2, seed

powder yield varied from 8.3 % to 43.4 % and was higher at a temperature of 80 °C. Its highest yield value was almost 6-fold greater than that achieved for peel. Yield is an important parameter for the development of new products on a large scale. It is expected that the use of the waste will generate high yields of the interesting product. In addition, humidity of the product should be reduced, providing a longer shelf life and a lower risk of microbiological contamination. However, it is not possible to assert that the seed drying process was less efficient, since the different matter has its own specific characteristics. Fig. 2 shows the response surfaces for both yields of melon peel and seed.

Fig. 2 indicates that the best drying yield for cantaloupe seed and peel occurred in the high temperature range, which confirms the higher importance of temperature over time in the yield of melon powder. This may be because a longer drying time is needed at low temperatures to achieve a better yield. Statistical analysis helped to identify the best drying conditions at an air temperature of 80 °C and drying time of 24 h, using high yield values as a criterion.

Residues characterization

The composition of residue powder is presented in Tab. 3. The moisture content of the samples showed significant variation ($p < 0.05$) between the powders, with peel flour showing a higher moisture than seed flour. This was possibly because the peel contained high amounts of carbohydrates, which are highly hygroscopic and might have captured more atmospheric humidity. The dried samples were stored in closed and sealed containers, protected against moisture. However, the geographic region in which the experiments were performed has high humidity, above 60 %, so it is possible that the presence of sugars would have an influence on hygroscopicity of the material. Moisture is an important parameter in drying processes since application in the food industry requires relatively low moisture content to develop products with a longer shelf life that are less prone

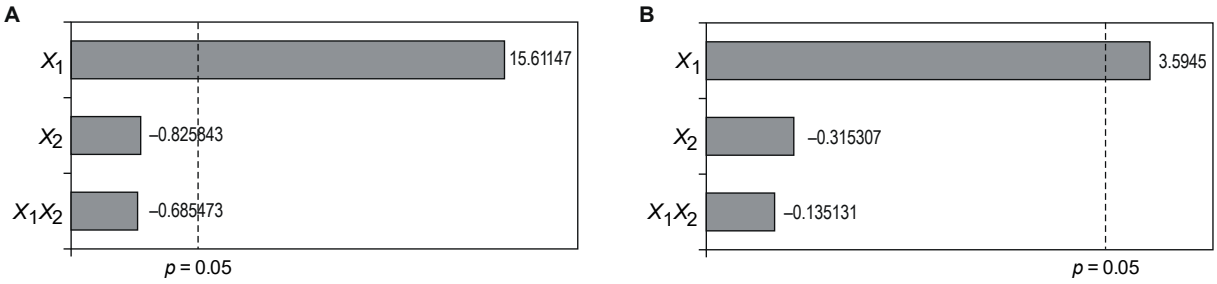

Fig. 1. Pareto's chart of the independent variables effects on the yield of melon seed and peel powders.

A – seed powder, B – peel powder.
X_1 – temperature, X_2 – time.

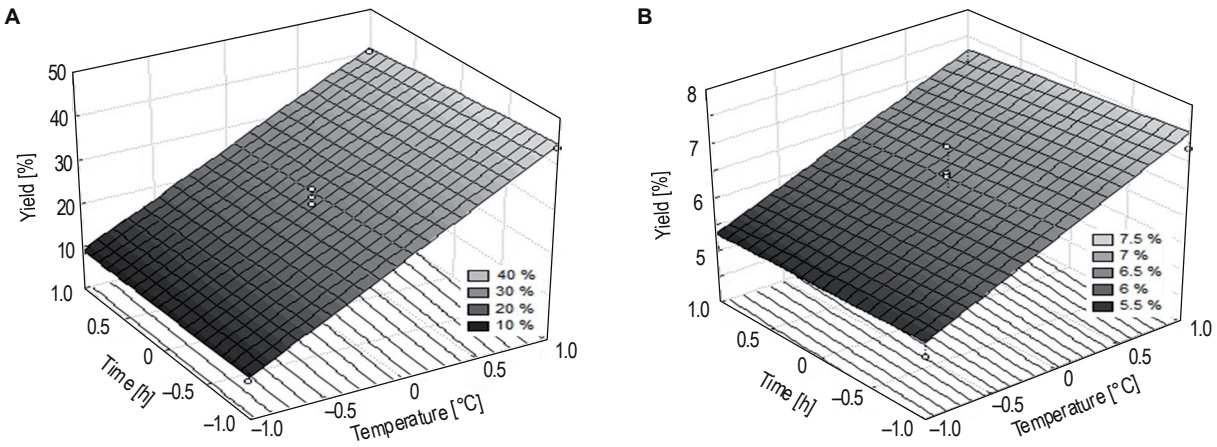

Fig. 2. Surface responses showing the yield of melon seed and peel powders as a function of the independent variables temperature and time.

A – seed powder, B – peel powder.

to microbiological contamination.

Similarly, the amount of ash present, which reflects the mineral content in food, differed significantly between powders and it was higher in the peel samples ($p < 0.05$). Previous studies reported lower values than those found in this study for melon seed, reaching 1.7 % ash, which may be the result of different ripening levels, and variations in climate and cultivar. It is important to note that high ash content is often observed in inedible parts of fruits, further re-inforcing the importance of the use of this waste. Also noteworthy is the high mineral content in residues submitted to drying [21].

Samples differed significantly in lipid content, with peel flour exhibiting lower values compared to seed flour ($p < 0.05$). A number of plant species accumulate oil in their seeds, acting as a reserve of energy during germination. One study found 246 $g \cdot kg^{-1}$ for melon seed flour, with a high potential for industrial, dietary, pharmacological and lubricant use, underscoring its economic importance [22]. The low lipid content in the peel suggests that this part can be used in food preparation without raising its lipid content.

By contrast, no differences were observed in relation to protein content, with samples exhibiting statistically similar values ($p > 0.05$). Proteins are essential nutrients in the human diet and the protein content in the flours was high, particularly in the seed flour. This information is important and it implies a possible application of this waste for protein enrichment of new foods, since demand for supplements has increased and this involves high costs. Considering the increase in the world population and diversity of diets, plant-based proteins have gained importance, exhibiting more economically viable production than their animal counterparts do. Melon seed powder reached significant values, and seeds in general are good sources of protein, since they store it in a concentrated form [23].

As a lignicellulosic residue, the insoluble fibres, such as cellulose, hemicellulose and lignin, were evaluated. Melon residues showed high contents of fibre, corroborating the results obtained with waste powders of other fruits, such as grape seed (47 %) [24] and mango peels (41 %) [25]. Different values (16 % fibre) were reported by Storck et al. [20] and Melo et al. [26] who found 19 % fibre in melon seeds.

Total dietary fibre is a complex fraction, often classified according to its solubility in water. The ratio of insoluble dietary fibre to soluble fibre varies according to the species and part of the plant it was extracted from. Plant wastes generally contain a larger proportion of insoluble fibre primarily due to their lignin and cellulose content [27]. Other studies also reported high insoluble fibre content in fruit waste. Grape skins contained 37 % insoluble fibre [28] and levels in the bagasse of different vegetables ranged from 18 % to 35 % [29]. To be considered a high-fibre product, a food must contain at least 6 g of dietary fibre per 100 g [30].

Tab. 3. Centesimal composition of the melon seed and peel powder after drying at 80 °C for 24 h.

Dry basis chemical composition	Seed powder	Peel powder
Moisture [%]	3.1 ± 0.9^{b}	8.4 ± 0.9^{a}
Ash [$g \cdot kg^{-1}$]	31.8 ± 1.3^{b}	85.6 ± 0.1^{a}
Lipid content [$g \cdot kg^{-1}$]	245.6 ± 2.8^{a}	36.3 ± 1.2^{b}
Protein [$g \cdot kg^{-1}$]	220.6 ± 1.3^{a}	175.3 ± 1.0^{a}
Insoluble fibre [$g \cdot kg^{-1}$]	453.2 ± 1.3^{a}	341.8 ± 2.7^{b}
Hemicellulose [$g \cdot kg^{-1}$]	79.1 ± 1.3^{a}	92.4 ± 1.9^{a}
Cellulose [$g \cdot kg^{-1}$]	350.2 ± 1.6^{a}	190.1 ± 1.7^{b}
Lignin [$g \cdot kg^{-1}$]	23.9 ± 1.9^{a}	59.3 ± 1.8^{a}

Values in the same row with same letters in superscript did not differ significantly ($p < 0.05$) in the Student's test. Results are means of 3 replicates with the respective standard deviation estimates.

The high content of fibres observed in the present study indicates that melon peel and seed flours can be included in the diet as a high-fibre food, in accordance with Brazilian legislation of Sanitary Supervision [31]. This result is remarkable since research demonstrates low fibre intake worldwide, which is associated with an increase in several chronic non-communicable diseases, while high fibre consumption is linked to the prevention of different pathologies [32, 33]. This suggests the potential use of these raw materials in new products as a source of fibre. The results indicate that this is a product with a high nutritional value, particularly in terms of protein content, and with a high fibre content.

Scanning electron microscopy analysis

Microscopic images of the peel flour are presented in Fig. 3. The material exhibited morphological differences under SEM. Particle size distribution was heterogenous in peel powder showing a granular and porous feature, with particles of different sizes and shapes (Fig. 3A) and oval-shaped voids, characteristic of the cantaloupe melon peel studied (Fig. 3B–3D). Microscopic images of the seed flour are presented in Fig. 4, showing a number of particles of different diameters (Fig. 4A). Surface roughness was the pri-

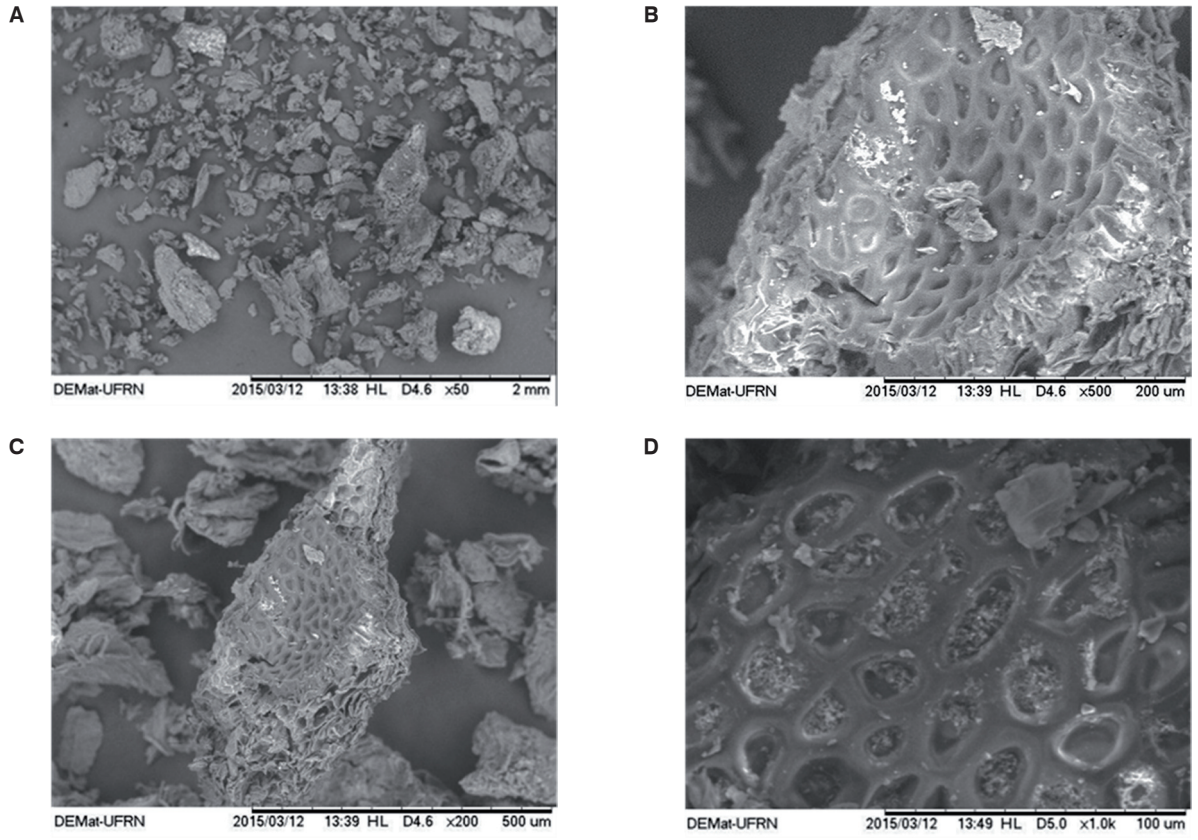

Fig. 3. Micrographs of the sample of melon peel powder.

A – magnification of 50x, B – magnification of 200x, C – magnification of 500x, D – magnification of 1 000x.

mary characteristic of heterogenous particles, with some exhibiting a smooth surface with high porosity and hollow particles (Fig. 4B, 4C).

Both samples contained structures possibly related to dietary fibre, which makes up the cell wall. Particle size distribution of the seed flour was heterogenous, with SEM images revealing a granular appearance and particles of different sizes and shapes, as well as circular and irregularly shaped fibrous particles. Generally, the surface of the powders was quite rough, exhibiting irregularities and porosity. More isolated structures were observed in the peel and clustered structures in the seed flour (Fig. 4D).

The presence of fibrous structures in the samples is an indication that these flours may resist the digestive process and bacterial fermentation. It could be observed that, in both samples, the morphological structures were possibly related to food fibres, components of the cell wall of the plant. Dietary fibre, especially cellulose, is particularly noteworthy in both the seed and peel samples, with micrographs indicating that the structures formed might largely be fibres.

In vitro prebiotic activity

The counts of *Bifidobacterium lactis* in the bioprocess using melon residues as a substrate are shown in Fig. 5. During the process of anaerobic fermentation for 24 h, the growth of bifidobacteria in different culture media was evaluated. Regarding the medium containing FOS, an internationally recognized prebiotic, an increase in bifidobacterial counts was observed from 4 h of fermentation, reaching a maximum in 14 h (12.8 log CFU·ml^{-1}). During fermentation, growth values for FOS were satisfactory, remaining above 9 log CFU·ml^{-1}. These counts are important, for instance, for colonization of the colon.

Similar results to those found with FOS were obtained with a medium containing melon seed flour. This substrate favoured the growth of lactic bacteria, especially bifidobacteria. A steady growth until 8 h of fermentation was observed and bacterial cells remained viable for up to 20 h of the submerged bioprocess. In contrast, the melon peel flour showed no viability of bifidobacteria, which can be interpreted as no prebiotic activity. As a substrate, melon peel did not stimulate the

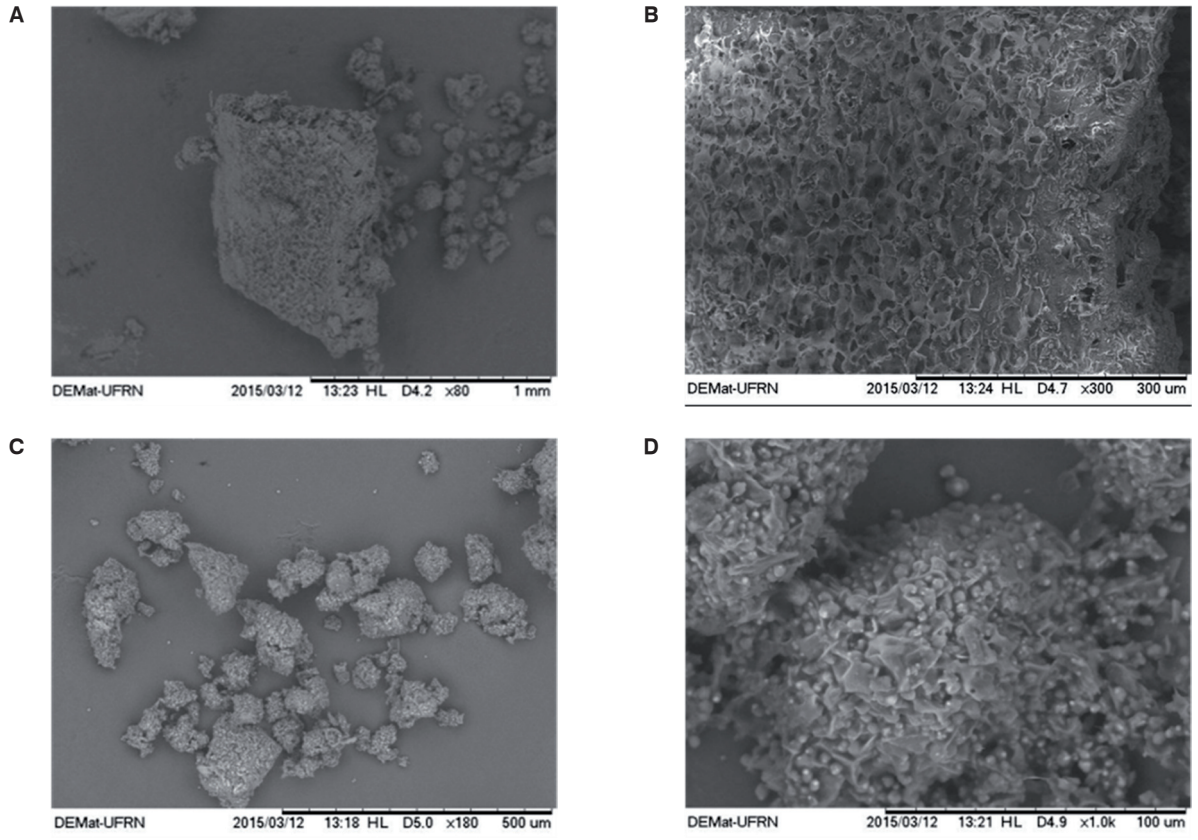

Fig. 4. Micrographs of the sample of melon seed powder.

A – magnification of 80x, B – magnification of 180x, C – magnification of 300x, D – magnification of 1 000x.

growth of the strain, and a decline in the bacterial growth curve was observed mainly after 2 h of fermentation. After 16 h, no viable cells could be counted on plates.

The pH values were also followed throughout the fermentation process (data not shown). For the culture medium containing FOS and the medium containing melon seed flour, the pH values were similar, ranging from 7.0 at the beginning of the fermentation to 5.0 at its end in 24 h. These data are in agreement with viability of bifidobacteria, since these microorganisms optimally grow in the range of 6.5–7.0, being unable to grow in environments with pH below 5.1 and above 8.0 [34]. Fermentation of melon peel led to high pH (above 7.0), especially after 10 h of fermentation. Possibly, there was competition with other lactic acid bacteria or presence of antimicrobial substances in the peel, which did not allow adequate heterofermentative metabolism of bifidobacteria.

A possible explanation for the fact that melon peel did not present prebiotic potential, since bifidobacteria did not grow, may be the lignin content that was higher than that found in seed. This may possibly be a limiting factor to the access of bifidobacteria to cellulose and hemicellulose. Moreover, the melon peel flour may contain toxins known as curcubitacins, identified in Cucurbitaceae, which may have had a toxic effect on bifidobacteria [35].

Regarding the melon seed flour, it is noteworthy that its composition comprising cellulose (35 %) and hemicellulose (7.9 %) could be more favoured for the growth of bifidobacteria that can excrete enzymes that degrade β-1-4-glucosidase bonds. Bifidobacteria metabolize many complex carbohydrates that are not hydrolysed by digestive enzymes in the gastrointestinal tract and reach the colon unabsorbed such as the non-digestible xylo-oligosaccharides, pectin-oligosaccharides and mannan-oligosaccharides [36]. In addition, *Bifidobacterium lactis* has been reported to metabolize xylo-oligosaccharide, thus indicating that xylan, a hemicellulose derived from the cell wall, can be a potential prebiotic candidate [37].

Being members of the group of heterofermentative bacteria, bifidobacteria use the pentose-phosphate pathway or Warburg-Dickens pathway for fermentation. Bifidobacteria are saccharolytic

and play an important role in fermentation of carbohydrates in the colon. Physiological data confirm that bifidobacteria can effectively ferment various complex carbohydrates, such as resistant starch, cellulose, hemicellulose, galactans, xylan, pectin and gums. They degrade these carbohydrates into low-molecular-weight oligosaccharides, forming monosaccharides by enzymes that hydrolyse carbohydrates, and then these monosaccharides are converted to hexoses in the fermentation pathway [38, 39].

The genome of bifidobacteria appears to reflect the adaptation to the environment of the human gastrointestinal tract, as evidenced by the presence of genes encoding a variety of carbohydrate-degrading enzymes, such as glycosyl hydrolases [40]. This may explain the activity of *B. lactis* in a medium containing melon seed flour, as the bacterium possibly degrades complex carbohydrates present in the substrate, such as cellulose, hemicellulose and pectins.

Metabolism of carbohydrates can vary considerably in the bifidobacteria strains. For instance, VAN DEN BROEK et al. [41] reported the fermentative characteristics of various species of the genus *Bifidobacterium* and identified strains that degraded cellobiose, such as *B. breve* UCC2003, *B. indicum* JCM1302, *B. dentium* JCM1195, *B. mongoliense* YIT10443 and *B. asteroids* JCM5821. These results are important and can explain the positive effect of melon seed on the growth of bifidobacteria, emphasizing that there is no evidence of oligosaccharides in melon residues. The highlight of the bifidobacterial genome is the potential presence of β-glucosidase (EC 3.2.1.21) due to the presence of the gene *cdlEFGC* in strains *B. animalis* subsp. *lactis* (AD101, BI-04 and HN019), the same strain as used in this study, and the cellulase enzyme (EC 3.2.1.4) in the strains *B. adolescentis* (ATCC 15703), *B. dentium* (ATCC 27678) and *B. longum* (ATCC 55813). The enzyme is able to hydrolyse cellobiose and cellodextrins [42].

Acid and bile tolerance are basic requirements in response to prebiotic criteria. The melon seed as a substrate in *B. lactis* fermentation showed an indication of prebiotic activity, and resistance to bile salts. According to Fig. 6, bacteria were viable during up to 8 h of fermentation with absorbance above 0.3. Strains showed 67% viability in a bile tolerance test after 6 h of fermentation.

These results indicate that the melon seed flour is a possible prebiotic ingredient, due to its potential to stimulate the growth of bifidobacteria and resist the action of bile salts for 8 h of fermentation. Characterization of new bacterial glycosyl hydrolases will be a new tool that will allow identifying substrates that can act as new prebiotics.

Carboxymethyl cellulase and filter paper activity

Cellulases are used in food, brewery and wine industries, animal feed, textile and laundry, pulp and paper industries, as well as in agriculture and for research purposes. Many cellulolytic products, such as hemicellulose and lignin, which are not suitable for human consumption, are converted into useful products by the help of microorganisms. Cellulolytic fungi are the widest utilized producers of cellulases due to easy handling and economically feasible processes as compared to other sources [43]. Cellulose is degraded by the hydrolytic action of a multi-component enzyme system and represents the key step for biomass

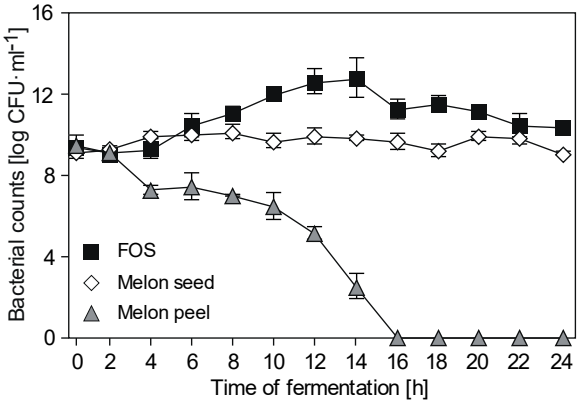

Fig. 5. Growth of *Bifidobacterium lactis* in submerged fermentation using melon peel, melon seed and fructooligosaccharide as substrate during 24 h.

FOS – fructooligosaccharide.

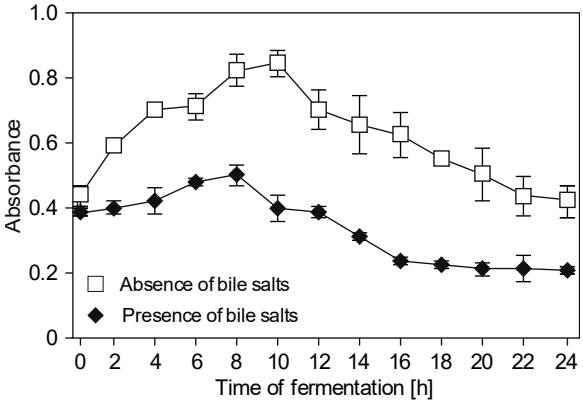

Fig. 6. Determination of bile salts tolerance of *Bifidobacterium lactis* during submerged fermentation using melon seed flour as a substrate.

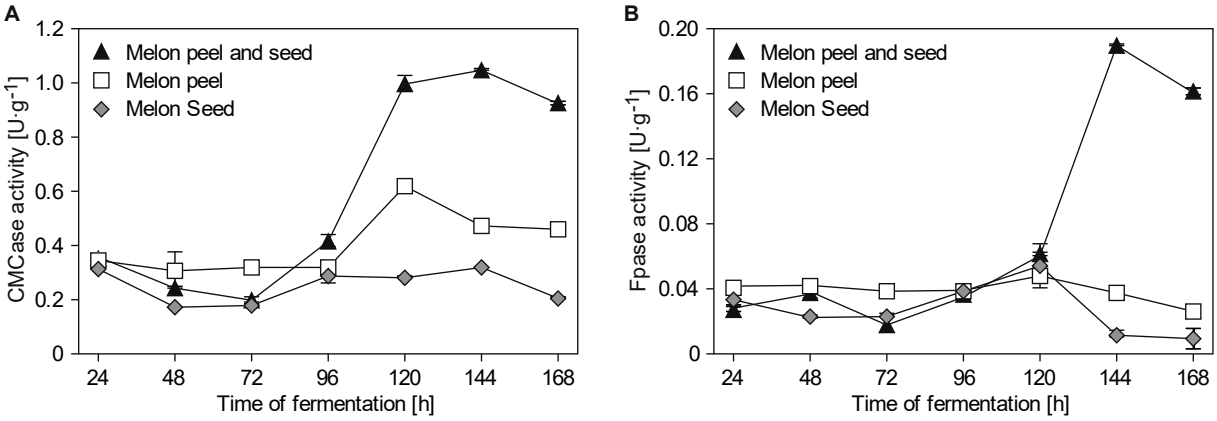

Fig. 7. Carboxymethyl cellulase and filter paper activities using different substrates in solid state fermentation by *Aspergillus oryzae* ATCC 9362.

A – carboxymethyl cellulase activity, B – filter paper activity.

conversion. The complete enzymatic hydrolysis of cellulosic materials requires different components, namely, endo-1,4-β-D-glucanase (EC 3.2.1.4), exo-1,4-β-D-glucanase (exocellulase, EC 3.2.1.74) and β-D-glucosidase (EC 3.2.1.21) [44]. Cellulolytic enzymes production is not only connected to the microorganism strain, but also to the techniques and substrates used in the process. Several factors may influence the production of the cellulolytic complex such as semi-solid or submerged fermentation, extraction mode, moisture, pH, the concentration of the inoculum, temperature and other factors [45].

The results of enzymatic activity showed that the microorganism used is a good producer of CMCase when both seed and peel were used as substrate, i.e. the enzyme was hydrolysing cellulose effectively since CMCase activity is related to the endo-cellulase activity. The maximum CMCase production rate occurred from 96 h up to 144 h reaching an activity value of 1.045 $U \cdot g^{-1}$ (Fig. 7). Our results are in agreement with those reported earlier, which stated that the maximum CMCase production by *Aspergillus japonicus* URM5620 was found in 120 h [46] and are somewhat different from another study, which reported cellulase production in 72 h by a fungus isolated from a rain forest [47].

The culture medium using the mixture of melon peel and seed (1:1) provided higher CMCase activity (1.045 $U \cdot g^{-1}$) than when using isolated media. YANG et al. [48] reported the activity of endoglucanase and FPase in submerged fermentation of *Coprinopsis cinerea* using banana peel as a substrate, and found activities of 0.48 $U \cdot ml^{-1}$ for endoglucanase and 0.19 $U \cdot ml^{-1}$ for FPase.

CMCase activity was also reported by MENEZES et al. [49], the results showing a lower activity of this enzyme, the largest activity of 0.06 $U \cdot ml^{-1}$ being produced by *Pleurotus tailandia* strain using sugarcane bagasse as substrate. In addition, they reported that *Aspergillus oryzae* produced a maximum CMCase activity of 0.966 $U \cdot ml^{-1}$, highlighting the ability of the latter to degrade cellulose. The possible mechanism behind the increase in the production of CMCase and FPase enzymes in the substrate containing the residue mixture (peel and seed flour) can be explained by the similarity of this medium to the natural habitat, in which there is a variety of components in the soil, for example, a higher amount of biomass, with an increase in carbon source and nitrogen source, favouring in vitro growth of *Aspergillus oryzae* mycelium.

As commented by NIGAM and SINGH [50], fungi are most important in SSF, as they can grow naturally in fruits, grains and agricultural waste, and growth conditions are very close to the natural conditions, i.e. in their natural habitat. The ability to grow in a low-pH medium and the ability of filamentous fungi to produce spores facilitate the inoculum preparation, besides storage of the cells in the vegetative form for long periods, are further features that make fungi attractive for SSC.

CONCLUSIONS

The use of cantaloupe melon (*Cucumis melo* L. var. *reticulatus)* peel and seed flours demonstrated their good nutritional value for application in food technology, with particularly high fibre and protein contents. Obtaining new prebiotic compounds

from lignocellulosic sources fermentation is an alternative to the food industry to obtain compounds with functional features, adding value to these residues, thus creating an alternative use of these, and reducing environmental impact. Also, we showed that using a mixture of melon peel and seed flour as a substrate for SSC by *Aspergillus oryzae* can produce CMCase (1.045 $U \cdot g^{-1}$) after 120 h cultivation at 30 °C and pH 5.5.

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